Editorial Comments:

1. The formatting from our prior draft has been maintained throughout our document. We continued to define all abbreviated terms upon first use and to use standard SI unit symbols (with a single space between numerical values and their limits).
2. We checked to ensure that our introduction included 1) the advantages over alternative techniques with applicable references to previous studies, 2) the context of this technique in the wider body of literature, and 3) information to help readers determine if the protocol is appropriate for their application.
3. Keywords/phrases were added to **lines 35 – 36**.
4. Non-imperative text found within the protocol was specified as a “Note” in **line 157**
5. Section 1.1 - Supplies have been specified without commercial language **line 125.**
6. Section 2.9 – Note added addressing how likely > 7 days are required prior to moving to experiments at **line 270.**
7. Section 3.2 – Edited to explain that a simple piece of tap is used to label the bottles. The label will not influence the mouse’s preference for one bottle or another, as the label cannot be seen by the mouse, **line 284.**
8. Section 3.8 – Wording and terminology was edited in this section to better clarify the steps of the protocol. More specifically, the term ‘vehicle’ was replaced with ‘control’. Additionally, we provided the volume of injectable solution used in our studies **line 309**
9. Section 3.9 – The text was clarified to better explain the dosing groups used and how subjects are assigned to their respective group.
10. Section 4.7 – Examples of possible blood collection techniques were provided, along with references for potential new methods, **line 411.**
11. Section 4 –Details were provided on when the drug was administered, **line 354**.
12. Figure titles added to each legend.
13. Figures 1 and 2 – groups and mean values were clarified, both in text and figure legends, **line 455**.
14. Figures 1 and 2- stars on horizontal lines explained in **line 475,484.**
15. The second paragraph of the representative results section was re-written to address any textual overlap with our previous publications, **line 429**.
16. References were reformatted to the appropriate style.
17. Acknowledgements section was added for all funding sources, **line 560**.
18. All commercial language was removed from the manuscript, but kept in the materials list (Including ‘Analox’ and ‘Jackson lab’).

**Reviewers' comments:**

**Reviewer #1:**

*1. Please comment on the influence of sex on assay results and which sex is preferred and/or what parameters influence the choice of which sex to use.*

All of the effects of a drug are not guaranteed to be observed in both genders due to the estrous cycle, genetic, and or underlying factors that can affect metabolism, etc.. As such, in drug discovery both female and male models are important for an evaluation of the true efficacy of the compound. Our studies with avermectins have shown that ivermectin and moxidectin can consistently and robustly reduce ethanol intake in both genders and across various paradigms. As such, the models discussed here are appropriate for both genders with no specific requirement pertaining to each gender.

*2. For drinking in the dark, are animals typically kept on a reverse light dark cycle? If mice are transferred from normal lighting to a reverse light cycle, how long should they be allowed to acclimate? I have seen some papers in the literature that allow two weeks. What is the timing of the light dark cycles (ie, when do lights come on and go off)?*

During drinking in the dark, lights are kept on a reverse light/dark cycle with lights off at 12:00 pm and on at 12:00 am. We agree that mice transferred from a standard light/dark cycle should spend 2 weeks adjusting to the new schedule. We have incorporated this information into **line 165-169.**

*3. I recommend not using the confusing term of "social" drinking for the 2BC procedure. Social, to many, implies drinking in a group, i.e., a social setting. While I understand that social can also mean moderate drinking, this term is confusing. It would be better to stick with a less confusing term like moderate or more clearly define what is meant by social.*

We agree that the term “social” drinking is misleading and have instead labeled it “moderate” drinking; see **line 77**.

*4. The terms bottles and sipper tubes are used interchangeably throughout the manuscript and this is confusing. Where you mean sipper tubes, that terminology should be strictly followed to avoid confusion.*

We have made these changes, see **line 140, 171, 240.**

*5. Section 2.6: This seems to be the only place you refer to "inverted sipper tubes". Do you really invert the sipper tubes to read them every time? If so this should be made clear and the term inverted sipper tubes should be mentioned throughout.*

The tubes themselves are situated on the cage top in an inverted position; we have added the information to **line 240.**

*6. Section 2.6: Standard scientific practice is to read the bottom (i.e., the lowest point) of the concave meniscus for water and ethanol solutions instead of the atypical "highest point" as written. Perhaps this is simply a semantic issue. It should either be clarified or made to conform to standard scientific practice.*

It should be noted that while it is standard scientific practice to read the lowest point of the concave meniscus, because the bottles remain in an inverted position during the measurement, we record the highest point of the meniscus. This was noted at **line 243**.

*7. Section 2.7: for clarity, change wording to "Body Weight Change".*

We have made this change, **line 250.**

*8. For the 2BC protocol, no details are provided about drug testing. When are drugs tested relative to (a) light dark cycle and (b) measurement of drinking? How many days is the assay performed with and without drug? Why 10% ethanol? Many use the assay at concentrations that vary from ~3-20%? Is 10% best for testing drugs? Can the assay be used at multiple concentrations of ethanol to test drug effects?*

Details were added at **line 280, 341, 470.**

*9. When referring to C57 or C57BL/6 it is critical to specify the substrain (e.g., C57BL/6J) because differences in substrain can make a huge difference in performance on these assays.*

Changes made at **line 475, 497.**

*10. Discussion, line 324: These models are NOT models of "human alcoholism". They are models of drinking behavior.*

Changes made at **line 549.**

*11. Discussion on important controls is missing. How can one be sure that a change in drinking due to a drug is not simply a toxic side effect, i.e., it is making the animals sick. Typically one looks at the effects of the drug on locomotor activity or some other parameter. Also, typically one would like to know if the drug effect is specific to alcohol drinking or consumption of other substances (e.g., sucrose). Also, one typically needs to know if the drug is having an effect via the intended mechanism or if it alters taste perception. Therefore, preference and consumption of saccharine and quinine is typically measured also.*

We have added this information along with a reference to better illustrate these additional investigations, **line 547.**

*12. A diagram illustrating the time course of these experiments would be very helpful.*

We have created 2 flow charts to illustrate the time course of these experiments (**figure 3 and 4**).

*13. Can animals be retested for several doses and/or different drugs? If so, what is the timing of those experiments? Is there a washout between drugs?*

Yes, animals can in fact be retested, assuming they are healthy and not too old. In our experience the washout between drugs can take around 1-2 weeks, **line 540.**

**Reviewer #2:**

Major comments

1. *Many mouse cages have filter tops, those must be removed. The tubes must be anchored in place, otherwise the mice can rattle the tubes causing fluid to leak. We use large black paperclips. I couldn't tell, it looked like they included catalog numbers, but these should be clearly labeled for the rubber stoppers, ball bearing sipper tubes, and pipettes.*

We have added this information at **line 223-225.**

1. *For drinking-in-the-dark it is better to use 10 ml pipettes for more accurate measurements within the shorter time period. For 2-bottle choice, 18 or 25 ml pipettes can be used.*

We have added this information at **line 147.**

1. *Line 129: The authors should explain that it is way easier to cut the plastic pipettes with a razor blade that is heated under a Bunsen burner, using a vice grip to handle the razor blade and gloves to avoid getting burned. Both the tip and the end need to be cut off.*

We have added this information at **line 142.**

*4. Line 132: It is way easier to insert the ball bearing sipper tube into the plastic pipette if the pipette is warmed under a heat gun. Using this method the sipper tube slips right in and gets sealed. The shrink tubing is extra precaution. The same heat gun can be used for the shrink tubing.*

We have added this information at **line 150.**

Minor comments:

1. *In the abstract second to last sentence should be binge drinking not binges drinking.*

We have edited this typo at **line 112.**